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Laboratory note

Synthesis and pharmacological properties of novel benzamide derivatives acting as ligands to the 5-hydroxytryptamine 4 (5-HT_4) receptor

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Abstract – A series of 4-amino-5-chloro-2-methoxy-*N*-(1-substituted piperidin-4-ylmethyl)benzamides was synthesized as novel gastroprokinetic agents. The affinity of these compounds for the 5-hydroxytryptamine 4 (5-HT_4) receptor was evaluated. Among these compounds, 4-amino-5-chloro-2-methoxy-*N*-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl]benzamide (**3f**, Y-34959) showed a higher affinity for the 5-HT_4 receptor (*K*i = 0.30 nmol/L) than for other receptors, and was confirmed to be a potent 5-HT_4 receptor agonist having contractile effects in the isolated guinea-pig ascending colon (EC_{50} = 1.2 nmol/L). In dogs, compound **3f** increased gastroprokinetic motility of both the gastric antrum and the ascending colon. In addition, this effect on the colon was inhibited by azasetron, a selective 5-HT_3 receptor antagonist, demonstrating that the effect of gastroprokinetic agents having 5-HT_3 receptor antagonism on the colon were reduced compared with that of selective 5-HT_4 receptor agonists. © 1999 Éditions scientifiques et médicales Elsevier SAS

5-HT_4 receptor agonist / 5-HT_4 receptor agonism / 5-HT_3 receptor antagonism / *N*-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl]benzamide / gastrointestinal motility

1. Introduction

Activation of the 5-hydroxytryptamine 4 (5-HT_4) receptor mediates a wide variety of effects in the central and peripheral nervous systems [1]. Benzamides (metoclopramide [2], cisapride [3], etc.) are used clinically as gastrointestinal motility stimulants. Recently, this gastroprokinetic effect is thought to be mediated by 5-HT_4 receptor agonism [4]. In addition, we also found the contractile potency in the isolated guinea-pig ascending colon and the binding affinity for the 5-HT_4 receptor were well correlated among these benzamides [5]. On the other hand, these benzamides show antagonistic activity against various receptors such as dopamine D_2, 5-HT_2 and 5-HT_3 receptors [6–9], and the antagonism should reduce the gastroprokinetic effect of 5-HT_4 receptor agonism and/or cause unfavourable side effects. For example, it is well known that the 5-HT_3 receptor antagonists induce constipation, and D_2 receptor antagonists cause central nervous system effects such as depression and extrapyramidal syndrome [10]. Therefore, the search for selective 5-HT_4 receptor agonists would be an important goal to develop novel useful gastrointestinal motility stimulants.

In the course of our synthetic studies on 5-HT_4 receptor agonists, we have found that the essential framework for selective 5-HT_4 receptor agonism was 4-amino-5-chloro-2-methoxy-*N*-(piperidin-4-ylmethyl)-benzamide with the polar side chain at the 1- position on the piperidine ring (compound **1** has a methylsulfonylaminoethyl group as a polar side chain as shown in *figure 1*) [11]. Modifications of the polar side chain of compound **1** led to the discovery of a novel gastroprokinetic agent, 4-amino-5-chloro-2-methoxy-*N*-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl]benzamide (**3f**, Y-34959). Compound **3f** was confirmed to be a selective 5-HT_4 receptor agonist. Herein, we describe the synthesis and the pharmacological data of the novel selective 5-HT_4 receptor agonist.

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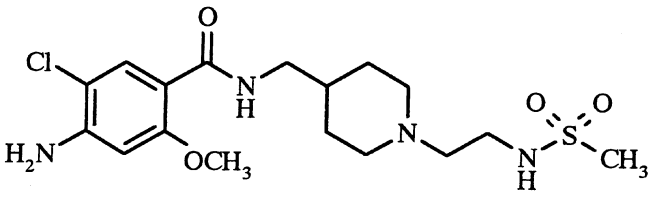


Figure 1. Chemical structure of compound **1**.

2. Chemistry

The general synthetic procedure used in this study is illustrated in *figure 2*. 1-Substituted-4-(*tert*-butoxycarbonylaminomethyl)piperidine derivatives (**2a**–**2f**) [12] were deprotected with hydrogen chloride in dioxane, affording the corresponding amines, which were coupled with 4-amino-5-chloro-2-methoxybenzoic acid using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (WSC HCl) and 1-hydroxybenzotriazole (HOBt) in the presence of triethylamine (TEA) to afford target benzamides (**3a**–**3f**), whose data for physicochemical properties and 5-HT_4 receptor affinities are listed in *table I*.

3. Pharmacological data and discussion

The affinity of compounds **3a**–**3f** for the 5-HT_4 receptor was determined as their ability to inhibit the binding of [^{3}H]GR113808 to the receptor. The agonistic activity of the candidate was evaluated as the contractile ability of the ascending colon in guinea-pigs. In in vivo studies, the gastrointestinal motility index of the candidate in conscious dogs was measured.

Binding affinities for the 5-HT_4 receptor of **1**, **3a**–**3f**, and 5-HT are listed in *table I*, where 5-HT is the reference compound. Phenylsulfonylamine derivative **3a** showed a higher affinity for the 5-HT_4 receptor than methylsulfonylamine derivative **1**. Next, we investigated the influence of other polar side chains. A benzamide group was selected, regarding the group of side chains, because an amide group is considered to be a bioisoster of the sulfonylamine group [13] and the introduction of the amide group is synthetically easier. Among the benzamide derivatives (**3b**–**3d**), an *N*-pentylbenzamide derivative **3d** showed the highest affinity for the 5-HT_4 receptor indicating that a bulky group is needed as a substituent on the piperidine ring at the 1- position and that a large pocket for the substituent should exist at the receptor. This consideration is supported by the López-Rodríguez report [14], which described a 5-HT_4 receptor mapping using an active analogue approach.

Next, the phenyl group was replaced by a 1-methylindole group. *N*-butyl-1-methylindole-3-carboxamide derivative **3e** showed a higher affinity than the corresponding compound **3c**. *N*-pentyl-1-methylindole-3-carboxamide derivative **3f** showed a subnanomolar affinity. These results suggest that the 1-methylindole-3-carboxamide group may interact with some amino acid

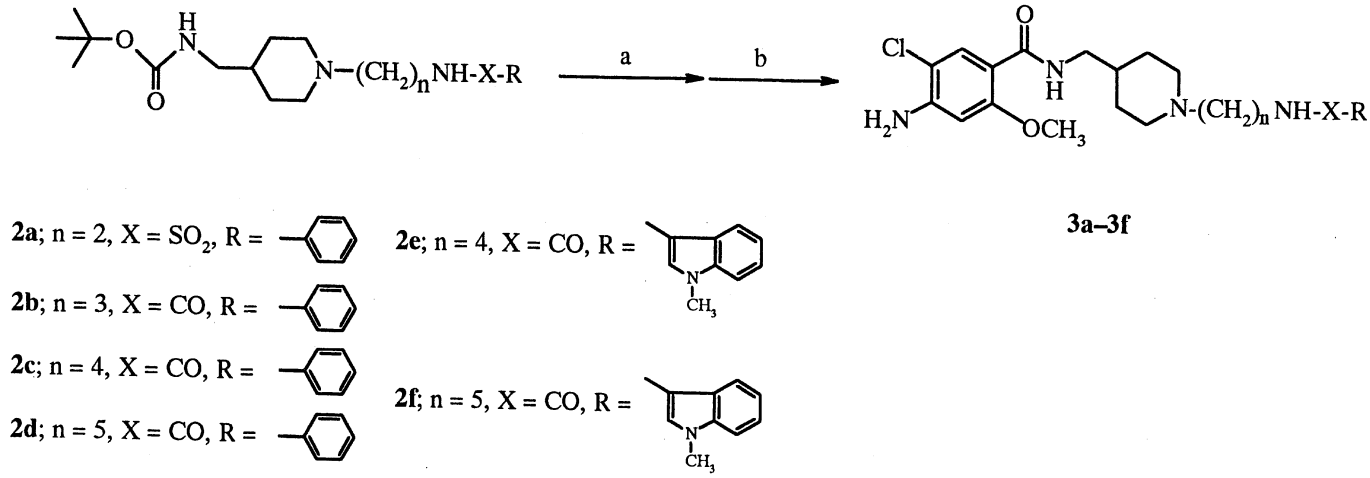
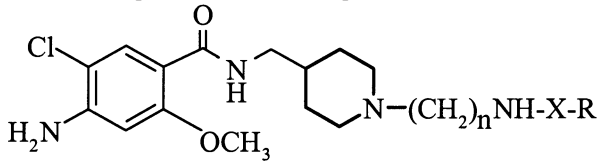
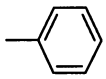
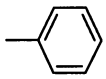
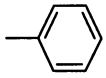
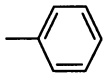
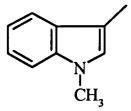
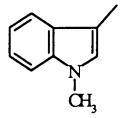


Figure 2. Synthesis of benzamides **3a**–**3f**. a: HCl in 1,4-dioxane. b: TEA, 4-amino-5-chloro-2-methoxybenzoic acid, WSC HCl, HOBt, DMF.

Table I. Physicochemical properties and 5-HT_4 receptor affinities of compounds **1** and **3a**–**3f**.



Compound	R	X	n	M.p. (°C)	Mass (m/z)	Formula	Binding affinity, Ki (nmol/L)[b]
1	CH_3	SO_2	2	177–178	418	$C_{17}H_{27}ClN_4O_4S$ 2 $C_2H_2O_4$[c]	9.6
3a		SO_2	2	Amorphous solid	480	$C_{22}H_{29}ClN_4O_4S$ 3/4H_2O	2.6
3b		CO	3	Amorphous solid	458	$C_{24}H_{31}ClN_4O_3$ H_2O	9.4
3c		CO	4	165–168	472	$C_{25}H_{33}ClN_4O_3$	4.0
3d		CO	5	122–124	486	$C_{26}H_{35}ClN_4O_3$ 1/2H_2O	1.7
3e		CO	4	Amorphous solid	525	$C_{28}H_{36}ClN_5O_3$ H_2O	1.6
3f		CO	5	161–163	539	$C_{29}H_{38}ClN_5O_3$ HCl	0.3
5-HT							130

[a]Elementary analyses were performed for C, H and N and were within ± 0.4% of the calculated values for formulae shown. [b]Each value is the mean from triplicate assays in a single experiment. [c]oxalate.

residues in the pocket of the 5-HT_4 receptor. Compound **3f** was devoid of significant affinities for other receptors, and produced concentration dependent contractions of the guinea-pig ascending colon (*table II*). Therefore, compound **3f** was confirmed to be a selective 5-HT_4 receptor agonist.

Finally, we examined effects of compound **3f** on gastrointestinal motility in conscious dogs in a postprandial state. When compound **3f** was given intravenously to dogs at 0.01 mg/kg, the motility of the gastric antrum and ascending colon was rapidly enhanced, as shown in *figure 3*, and the motility index of the gastric antrum and ascending colon increased. In addition, we tested the influence of azasetron [15], a selective 5-HT_3 receptor antagonist, on the stimulated gastrointestinal motility by a selective 5-HT_4 receptor agonist compound **3f**. Azasetron inhibited the increase in the colonic motility index caused by compound **3f**. In contrast, azasetron did not influence the increase of the antral motility index (*figure 4*). These results demonstrated that gastroprokinetic agents which have 5-HT_3 receptor antagonism would be less effective in increasing the motility of the ascending

Table II. Binding profiles and 5-HT_4 receptor agonistic activity of compound **3f**.

Binding affinity[a], *Ki* (nmol/L)					5-HT_4 receptor agonistic activity	
D_2	5-HT_{1A}	5-HT_2	5-HT_3	5-HT_4		
rat striatum	rat hippocampus	rat cerebral cortex	rat striatum	guinea-pig striatum	EC_{50} (nmol/L)[c]	Maximal response (%)[d]
[^{3}H]spiperone	[^{3}H]8-OH-DPAT	[^{3}H]ketanserin	[^{3}H]granisetron	[^{3}H]GR113808	1.2 ± 0.3	6.3 ± 0.1
> 1 000[b]	> 1 000[b]	110	> 1 000[b]	0.3		

[a]Each value is the mean from triplicate assays in a single experiment. [b]IC_{50} value. [c]EC_{50} values (mean ± SE) was determined by linear regression. [d]A percentage (mean ± SE) of the contraction caused by methacholine (30 μmol/L).

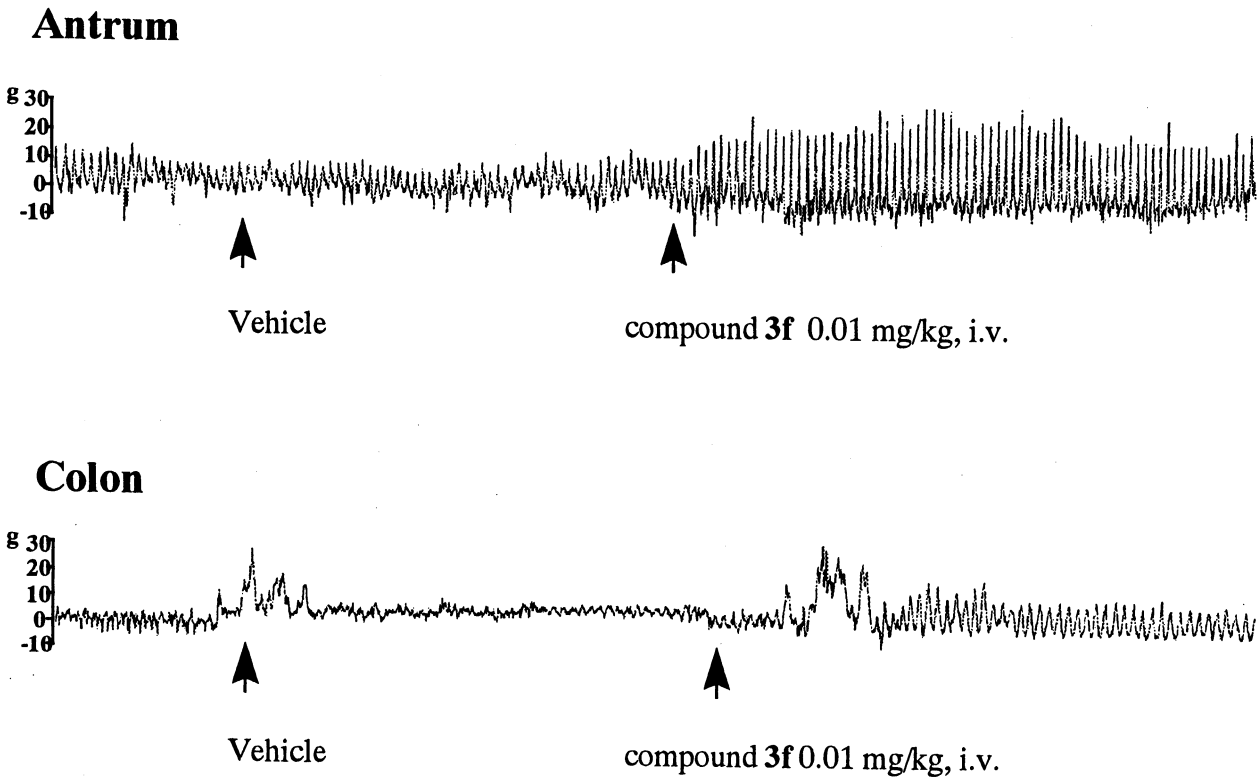


Figure 3. Typical tracings of the effect of compound **3f** on gastrointestinal motility in conscious dogs in a postprandial state.

colon than selective 5-HT_4 receptor agonists and that selective 5-HT_4 receptor agonists should be able to enhance both upper and lower gastrointestinal motility.

4. Conclusion

We described the synthesis, and affinity for the 5-HT_4 receptor, of a series of benzamides. Among them, 4-amino-5-chloro-2-methoxy-*N*-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl]benzamide (**3f**, Y-34959) was found to be a selective 5-HT_4 receptor agonist. Compound **3f** increased the gastrointestinal motility of both the gastric antrum and the ascending colon in conscious dogs in a postprandial state. Azasetron, a selective 5-HT_3 receptor antagonist, inhibited the increase of the colonic motility index caused by compound **3f** without influencing the increase of the gastric motility index. Based on our results, we proposed that the selec-

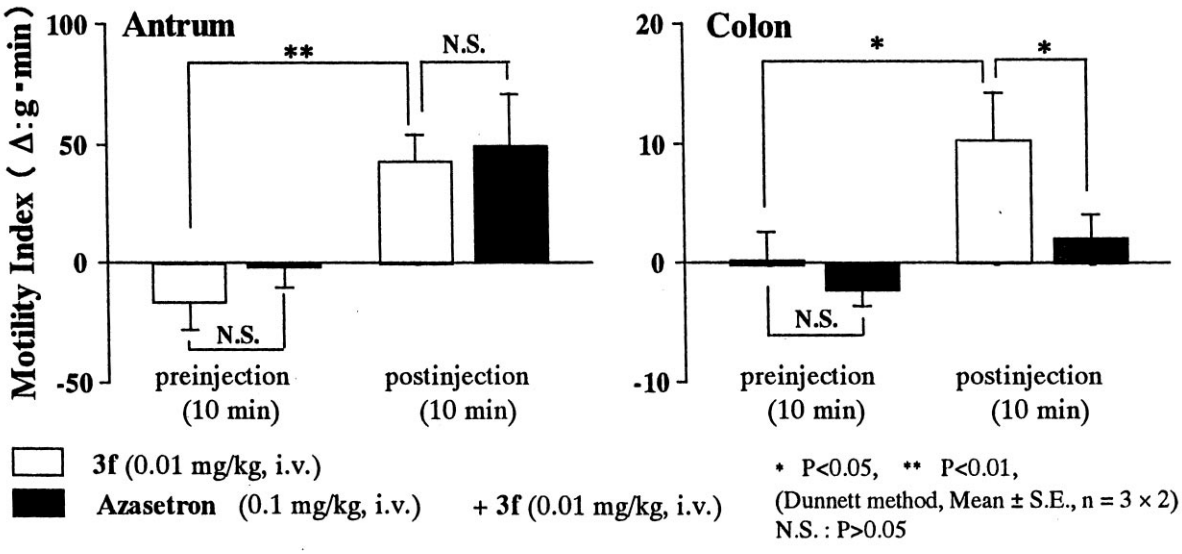


Figure 4. Effect of azasetron on the increase in the gastrointestinal motility of gastric antrum and ascending colon caused by compound **3f**.

tive 5-HT_4 receptor agonists were novel gastrointestinal motility stimulants which can enhance both upper and lower gastrointestinal motility with few side effects.

5. Experimental protocols

5.1. Chemistry

All melting points were measured in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on JEOL JNM-EX270 spectrometers and chemical shifts are expressed in ppm with tetramethylsilane (TMS) as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), br-s (broad singlet) and m (multiplet). Mass spectra (MS) were taken on JEOL JMS-O1SG spectrometers. Elementary analysis was performed for C, H, N and were within ± 0.4% of the calculated values. Silica-gel plates (Merck F254) and silica gel 60 (Merck, 70–230 mesh) were used for analytical and preparative column chromatography, respectively.

5.1.1. General procedure for the preparation of **3a**–**3f**

5.1.1.1. 4-Amino-5-chloro-2-methoxy-N-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl] benzamide hydrochloride **3f**

A mixture of **2f** (5.7 g, 13 mmol) and 4 mol/L hydrogen chloride in 1,4-dioxane (Aldrich) (60 mL) was stirred under ice-cooling for 1 h. After evaporation, the residue was washed with $CHCl_3$, and the resulting compound was dissolved in 80 mL of dimethylformamide (DMF). To the solution were added triethylamine (TEA) (5.7 mL, 41 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (2.6 g, 13 mmol) and 1-hydroxybenzotriazole (HOBt) (1.9 g, 14 mmol). The reaction mixture was stirred at room temperature for 1 h and then 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (WSC) (2.7 g, 14 mmol) was added under ice-cooling. Stirring was continued overnight at room temperature. After evaporation, 5% aqueous sodium bicarbonate was added to the residue and extracted with $CHCl_3$. The extract was washed with brine and dried over anhydrous magnesium sulfate. After evaporation in vacuo, the residue was chromatographed on silica gel eluting with $CHCl_3$–MeOH–NH_4OH (100:10:1) and treated with an alcoholic solution of hydrogen chloride. The precipitates were collected and recrystallized from ethanol to give **3f** (3.1 g, 43%); ^{1}H-NMR (DMSO-d_6) δ: 1.25–1.41 (2H, m), 1.47–1.62 (4H, m), 1.67–1.95 (4H, m), 2.80–2.93 (2H, m), 2.96 (2H, br-s), 3.17 (2H, br-s), 3.26 (2H, dd, J = 5.9, 13 Hz), 3.40–3.46 (2H, m), 3.82 (3H, s, CH_3N), 3.83 (3H, s, CH_3O), 5.95 (2H, s, Ar-NH_2), 6.50 (1H, s, Ar-3-H), 7.13 (1H, t, J = 6.6 Hz, ind-5-H), 7.20 (1H, t, J = 6.6 Hz, ind-6-H), 7.46 (1H, d, J = 7.9 Hz, ind-7-H), 7.66 (1H, s, ind-2H), 7.93 (1H, t, J = 5.3 Hz, $CONHCH_2$), 8.02 (1H, s, Ar-6-H), 8.14 (1H, d, J = 7.9 Hz, ind-4-H), 10.20 (1H, br-s, NHCO).

5.1.1.2. 4-Amino-5-chloro-2-methoxy-N-[1-[2-(phenylsulfonyl)aminoethyl]piperidin-4-yl]benzamide **3a**

Similarly to **3f**, **3a** was prepared starting from **2a** (1.6 g, 3.9 mmol), hydrogen chloride in 1,4-dioxane (11 mL), TEA (1.1 mL, 12 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (0.78 g, 3.9 mmol), HOBt (0.58 g, 4.3 mmol), DMF (20 mL), and WSC (0.82 g, 4.3 mmol) to give **3a** (0.55 g, 29%); ^{1}H-NMR ($CDCl_3$) δ: 1.20 (2H, dd, *J* = 11, 15 Hz), 1.53–1.67 (3H, m), 1.86 (2H, t, *J* = 9.9 Hz), 2.36 (2H, t, *J* = 5.9Hz), 2.59 (2H, d, *J* = 11 Hz), 2.98 (2H, t, *J* = 6.6 Hz), 3.31 (2H, t, *J* = 6.0 Hz), 3.92 (3H, s, CH_3O), 4.39 (2H, br-s, Ar-NH_2), 6.31 (1H, s, Ar-3-H), 7.48–7.60 (3H, m, Ar-H), 7.60 (1H, t, *J* = 6.0 Hz, $CONHCH_2$), 7.84 (2H, dd, *J* = 2.0, 6.6 Hz, Ar-H), 8.11 (1H, s, Ar-6-H).

5.1.1.3. 4-Amino-N-[1-(3-benzoylaminopropylpiperidin)-4-ylmethyl]-5-chloro-2-methoxybenzamide **3b**

Similarly to **3f**, **3b** was prepared starting from **2b** (1.0 g, 2.7 mmol), hydrogen chloride in 1,4-dioxane (11 mL), TEA (1.1 mL, 8.1 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (0.54 g, 2.7 mmol), HOBt (0.41 g, 3.0 mmol), DMF (15 mL), and WSC (0.58 g, 3.0 mmol) to give **3b** (0.10 g, 11%), ^{1}H-NMR ($CDCl_3$) δ: 1.44 (2H, dd, *J* = 12, 22 Hz), 1.72–189 (5H, m), 2.13 (2H, t, *J* = 11 Hz), 2.68 (2H, t, *J* = 6.0 Hz), 3.15 (2H, d, *J* = 12 Hz), 3.32 (2H, t, *J* = 6.6 Hz), 3.56 (2H, dd, *J* = 3.4, 12 Hz), 3.86 (3H, s, CH_3O), 4.42 (2H, s, Ar-NH_2), 6.29 (1H, s, Ar-3-H), 7.37–7.44 (3H, m, Ar-H), 7.74 (1H, t, *J* = 6.0 Hz, $CONHCH_2$), 7.84 (2H, dd, *J* = 2.0, 8.0 Hz, Ar-H), 8.10 (1H, s, Ar-6-H), 8.28 (1H, br-s, CONH).

5.1.1.4. 4-Amino-N-[1-(4-benzoylaminobutyl)piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide **3c**

Similarly to **3f**, **3c** was prepared starting from **2c** (0.86 g, 2.2 mmol), hydrogen chloride in 1,4-dioxane (9 mL), TEA (0.90 mL, 6.6 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (0.44 g, 2.2 mmol), HOBt (0.32 g, 2.4 mmol), DMF (15 mL), and WSC (0.46 g, 2.4 mmol). The resulting solid was recrystallized from ethanol–isopropanol to give **3c** (0.20 g, 19%); ^{1}H-NMR ($CDCl_3$-CD_3OD) δ: 1.32–1.56 (4H, m), 1.64–1.89 (5H, m), 2.13 (2H, t, *J* = 11 Hz), 2.68 (2H, t, *J* = 6.0 Hz), 3.21 (2H, d, *J* = 12 Hz), 3.39 (2H, t, *J* = 6.6 Hz), 3.43 (2H, t, *J* = 6.6 Hz), 3.91 (3H, s, CH_3O), 4.45 (2H, br-s, Ar-NH_2), 6.35 (1H, s, Ar-3-H), 7.33–7.42 (3H, m, Ar-H), 7.74 (1H, t, *J* = 6.0 Hz, $CONHCH_2$), 7.78 (2H, dd, *J* = 2.0, 8.0 Hz, Ar-H), 8.01 (1H, s, Ar-6-H).

5.1.1.5. 4-Amino-N-[1-(5-benzoylaminopentyl)piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide **3d**

Similarly to **3f**, **3d** was prepared starting from **2d** (1.7 g, 4.2 mmol), hydrogen chloride in 1,4-dioxane (17 mL), TEA (1.7 mL, 13 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (0.84 g, 4.2 mmol), HOBt (0.62 g, 4.6 mmol), DMF (20 mL), and WSC (0.88 g, 4.6 mmol). The resulting solid was recrystallized from ethyl acetate to give **3d** (1.4 g, 72%); ^{1}H-NMR ($CDCl_3$) δ: 1.31–1.49 (4H, m), 1.50–1.85 (7H, m), 1.91 (2H, t, *J* = 12 Hz), 2.32 (2H, t, *J* = 7.8 Hz), 2.92 (2H, d, *J* = 12 Hz), 3.30 (2H, t, *J* = 6.0 Hz), 3.34 (2H, dd, *J* = 7.3, 13 Hz), 3.88 (3H, s, CH_3O), 4.46 (2H, s, Ar-NH_2), 6.30 (1H, s, Ar-3-H), 6.36, (1H, br-s, CONH), 7.33–7.42 (3H, m, Ar-H), 7.70 (1H, br-s, $CONHCH_2$), 7.77 (2H, dd, *J* = 7.3 Hz, Ar-H), 8.09 (1H, s, Ar-6-H).

5.1.1.6. 4-Amino-5-chloro-2-methoxy-N-[1-[4-(1-methylindol-3-ylcarbonylamino)butyl]piperidin-4-ylmethyl]benzamide **3e**

Similarly to **3f**, **3e** was prepared starting from **2e** (1.4 g, 3.2 mmol), hydrogen chloride in 1,4-dioxane (14 mL), TEA (1.3 mL, 9.6 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (0.66 g, 3.2 mmol), HOBt (0.47 g, 3.5 mmol), DMF (30 mL), and WSC (0.67 g, 3.5 mmol) to give **3e** (0.63 g, 38%); ^{1}H-NMR ($CDCl_3$) δ: 1.24 (2H, dd, *J* = 11, 15 Hz), 1.30–1.72 (9H, m), 1.91 (2H, t, *J* = 9.9 Hz), 2.38 (2H, t, *J* = 6.6 Hz), 2.93 (2H, d, *J* = 11 Hz), 3.29 (2H, t, *J* = 6.6 Hz), 3.50 (2H, dd, *J* = 6.0, 12 Hz), 3.81 (3H, s, CH_3N), 3.87 (3H, s, CH_3O), 4.35 (2H, br-s, Ar-NH_2), 6.20 (1H, br-s, CONH), 6.26 (1H, s, Ar-3-H), 7.21–7.34 (2H, m, ind-5,6-H), 7.63 (1H, s, ind-2-H), 7.70 (1H, t, *J* = 3.2 Hz, $CONHCH_2$), 7.89 (1H, d, *J* = 8.0 Hz, ind-7-H), 7.92 (1H, d, *J* = 8.0 Hz, ind-4-H), 8.10 (1H, s, Ar-6-H).

5.2. Pharmacology

5.2.1. 5-HT$_4$ receptor binding

Male Hartley guinea-pigs (Japan SLC, Ltd., Shizuoka, Japan) were killed by cervical dislocation and the striatum was separated from each brain. The striatum was homogenized in 15 volumes of 50 mmol/L ice-cold HEPES buffer (pH 7.4) with Polytron PT-10 and then centrifuged at 35 000 *g* for 20 min. The resulting pellet was resuspended in the HEPES buffer and finally diluted to the appropriate concentration for assay (6 mg wet weight per assay tube). This suspension was used as the tissue preparation. Assay tubes contained 50 mL of HEPES buffer or a solution of the test agents, 50 mL solution of [^{3}H]GR113808 (Amersham International, UK) to give a final concentration of 0.1 nmol/L and 900 mL of tissue preparation. Each tube was incubated for 30 min at 37 °C and the reaction was terminated by rapid filtration through a Whatmann GF/B filter (presoaked in 0.01% v/v polyethyleneimine) followed by washing with 1 × 4 mL of ice-cold HEPES buffer. Then

the filter was placed in 3 mL of scintillant and the radioactivity was determined by scintillation counting in a Beckman model LS3801 scintillation counter. Non specific binding was defined in the presence of unlabelled GR113808 to give a final concentration of 1 μmol/L. The IC_{50} value was determined by non-linear regression of the displacement curve, and the *K*i value was calculated according to the formula ($Ki = IC_{50}/(1 + L/Kd)$), where L is the concentration of radioligand and *K*d is the dissociation constant of the radioligand.

5.2.2. Binding to other receptors

The binding studies of D_2 [16], 5-HT_{1A} [17], 5-HT_2 [18], and 5-HT_3 receptor [19] were carried out according to the previously published methods.

5.2.3. 5-HT_4 receptor agonism

Four male Hartley guinea-pigs (Japan SLC, Ltd., Shizuoka, Japan) were killed by cervical dislocation and the ascending colon was removed. The longitudinal muscle layer was separated from the underlying circular muscle. Each muscle strip preparation of about 2.5 cm was individually mounted vertically for isotonic measurement into a tissue bath containing 10 mL Tyrode solution. This solution was kept at 37 °C and gassed with 95% O_2, 5% CO_2. The strips were subjected to a preload of 1 g and allowed to stabilize for 20 min. After stabilization, the response of the longitudinal muscle to 30 μmol/L methacholine was measured. Agonist concentration-effect curves were constructed using sequential dosing, leaving 15 min between doses. A 15 min dosing cycle was required to prevent desensitization. The agonist was left in contact with a preparation until the response had reached a maximum, the preparation was then washed. Forty minutes was left between the determination of concentration-effect curves. GR113808 (10 nmol/L) was incubated for 10 min before repeating the agonist concentration-effect curves. After each determination of a concentration-effect curve, 30 μmol/L of methacholine was added to the tissue bath again. All responses were expressed as a percentage of the mean of the two contractions induced by 30 μmol/L methacholine. The EC_{50} value, the concentration causing 50% of the maximal response, was determined by linear regression analysis.

5.2.4. Gastrointestinal motility

Three mongrel dogs were used. Under pentobarbital anaesthesia, the abdomen was opened, and strain gauge transducers (F-12SSH, Star Medical, Tokyo, Japan) were sutured onto the serosa of the stomach and colon in a manner to detect circular muscle contraction. The transducers were placed at the greater curvature of the gastric antrum, 5 cm proximal to the pyloric ring, and at the colon, 10 cm distal to the ileo-colic junction. The animals were then allowed more than 2 weeks to recover from the surgery. To measure the gastrointestinal motor activity, the signals from the gastric antrum and colon were recorded on a recording system (ESC-2000, Star medical, Tokyo, Japan) every 100 ms by a telemetry system (DAS-800T, Star medical, Tokyo, Japan). The motility index of contractile activity, shown as an area under contraction wave, was calculated both in the gastric antrum and in the colon by means of a program (Peaks, AD Instruments, Australia). Measurements of gastrointestinal motility were performed in a postprandial state. Approximately 150 min after feeding of a dry type meal (200 g), azasetron (0.1 mg/kg) or vehicle was given to the dog intravenously. Ten minutes later, test compound was injected intravenously. The motility index during 10 min before and after injection of the test compound was determined, and data were expressed as a difference between the motility index before and that after the injection of the test compound. Investigation consisted of six experiments in three dogs. Statistical analysis of data was performed by means of the Dunnett method.

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References

[1] Eglen R.M., Wong E.F., Dumuis A., Bockaert J., Trends Pharmacol. Sci. 16 (1995) 391–398.

[2] Harrington R.A., Hamilton C.W., Brogden R.N., Linkewich J.A., Romankiewicz J.A., Heel R.C., Drugs 25 (1983) 451–494.

[3] McCallum R.W., Prakash C., Campoli-Richards D.M., Goa K.L., Drugs 36 (1988) 652–681.

[4] Bockaert J., Fozard J.R., Dumuis A., Clarke D.E., Trends Pharmacol. Sci. 13 (1992) 141–145.

[5] Sato N., Asano K., Haga K., Arita M., Jpn. Pharmacol. Ther. 25 (1997) 383–387.

[6] Turconi M., Schiantarelli P., Borsini F., Rizzi C.A., Ladinsky H., Donetti A., Drugs Future 16 (1991) 1011–1026.

[7] Cushing D.J., Cohen M.L., J. Pharmacol. Exp. Ther. 264 (1993) 193–199.

[8] Yoshida N., Omoya H., Furukawa K., Ito T., Karasawa T., Arch. Int. Pharmacodyn. Ther. 300 (1989) 51–67.

[9] Karasawa T., Yoshida N., Furukawa K., Omoya H., Ito T., Eur. J. Pharmacol. 183 (1990) 2181.

[10] Fernandez A.G., Roberts D.J., Drugs Future 16 (1991) 885–892.

[11] Itoh K., Kanzaki K., Ikebe T., Kuroita T., Tomozane H., Sonda S., Sato N., Haga K., Kawakita T., Eur. J. Med. Chem. 34 (1999) 977–989.

[12] Kawakita T., Kuroita T., Murozono T., Hakira H., WO 95/26953 Chem. Abstr. 124 (1996) 14913.

[13] Thornber C.W., Chem. Soc. Rev. 8 (1979) 563–580.

[14] López-Rodríguez M.L., Morcillo M.J., Benhaú B., Rosado M.L., J. Comput. -Aided Mol. Des. 11 (1997) 589–599.

[15] Kawakita T., Kuroita T., Yasumoto M., Sano M., Inaba K., Fukuda T., Tahara T., Chem. Pharm. Bull. 40 (1992) 624–630.

[16] Creese I., Schneider R., Snyder S.H., Eur. J. Pharmacol. 46 (1977) 377–381.

[17] Hall M.D., El Mestikawy S., Emerit M.B., Pichat L., Hamon M., Gozlan H., J. Neurochem. 44 (1985) 1685–1696.

[18] Leysen J.E., Niemegeers C.E., Van Nueten J.M., Laduron P.M., Mol. Pharmacol. 21 (1982) 301–314.

[19] Nelson D.R., Thomas D.R., Biochem. Pharmacol. 38 (1989) 1693–1695.